ELSEVIER

Cell Calcium 43 (2008) 236–249

cell calcium

www.elsevier.com/locate/ceca

Calcium signal transmission in chick sensory neurones is diffusion based

William Coatesworth, Stephen Bolsover*

Department of Physiology, University College London, United Kingdom

Received 22 March 2007; accepted 18 May 2007
Available online 12 July 2007

Abstract

In many cells, the cytosol is an excitable medium through which calcium waves propagate by calcium induced calcium release (CICR). Many labs. have reported CICR in neurones subsequent to calcium influx through voltage gated channels. However, these have used long depolarizations. We have imaged calcium within chick sensory neurones following 50 ms depolarizations. Calcium signals travelled rapidly throughout the cell, such that changes at the cell centre were delayed by 24 ms compared to regions 3 μm from the plasma membrane. The nuclear envelope imposed a delay of 9 ms.

A simple diffusion model with few unknowns gave good fits to the measured data, indicating that passive diffusion is responsible for signal transmission in these neurones. Simulations run without indicator dye did not reveal markedly different spatiotemporal dynamics, although concentration changes were larger. Simulations of calcium changes during action potentials revealed that large calcium transients occurring in the cytosol close to the nucleus are significantly attenuated by the nuclear envelope.

Our results indicate that for the brief depolarisations that neurones will experience during normal signal processing calcium signals are transmitted by passive diffusion only. Diffusion is perfectly capable of transmitting the calcium signal into the interior of nerve cell bodies, and into the nucleoplasm.

Keywords: Calcium; Diffusion; Nucleus; Nuclear envelope; Neurone; Chick; Sensory neurone; Confocal imaging; Modelling

1. Introduction

The diffusion of calcium through cytosol is the basic mechanism by which calcium ions entering the cytosol at one location exert their effects on remote targets. Depending on the cell type, more complex and cell-specific mechanisms may amplify and modify simple diffusion. In particular, a high density of ryanodine receptors, or a raised cytosolic concentration of inositol trisphosphate, may allow the cytoplasm to function as an excitable medium, propagating a travelling calcium signal at speeds of about 50 $\mu m\ s^{-1}$ in a regenerative manner [1,2].

Some neurones, including hippocampal and neocortical pyramidal neurones, generate regenerative calcium waves during repetitive synaptic stimulation. However, these waves are proposed to cause changes in synaptic strength and cellular architecture rather than playing a role in routine synaptic transmission [3,4]. For the majority of the time and in the majority of neurones the role of relaying to intracellular components and organelles, in particular to mitochondria and the nucleus, information about electrical activity and agonist-evoked calcium entry at the plasma membrane, is likely to be fulfilled by simple diffusion of calcium alone.

A popular view of calcium signalling regards diffusion, unaided by regenerative processes, as allowing only short range signal propagation. In a review in 1994, Kasai and Petersen [5] estimated that the range of action (that is, the length constant λ) for diffusion-limited calcium signals would be less than 4 μm. This argument suggests that internal structures may be essentially insulated from events at the plasma membrane.

* Corresponding author at: Department of Physiology, University College London, Gower Street, London WC1E 6BT, United Kingdom.
Tel.: +44 20 7679 6564; fax: +44 20 7383 7005.
E-mail address: s.bolsover@ucl.ac.uk (S. Bolsover).

0143-4160/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ceca.2007.05.016

A particular question arises over the properties of the nuclear envelope. Some authors have argued that the nuclear envelope can adopt a conformation that is essentially impermeable to passive diffusion, allowing permanent concentration gradients between cytosol and nucleoplasm to be maintained [6–9]. Other authors have argued that nuclear pores offer an easy pathway for calcium diffusion so that the nuclear envelope delays signal propagation by only a few milliseconds [10,11]. The behaviour of the nuclear envelope is particularly important for an understanding of how electrical activity at the plasma membrane affects gene transcription in the nucleus through nuclear resident targets such as calmodulin dependent kinase IV [12].

Of necessity, measurement of calcium spatial dynamics requires introducing a calcium indicator into the cell. Indicators are usually mobile, and therefore will contribute to the propagation of the calcium signal, that is, diffusion of calcium:indicator complex will occur in addition to all the processes that take place in the unmanipulated cell. Use of an immobile or slowly diffusing indicator is no solution since this will have the opposite effect of slowing propagation below that occurring in an unmanipulated cell.

Our approach in this study has been to take a simple example and generate a mathematical description of simple diffusion with as few unknowns as possible. We have replated sensory neurones from dorsal root ganglia the day before the experiment to generate a simple topology without neurites. We have used the modelling environment Virtual Cell (http://www.nrcam.uchc.edu/) to create a three-dimensional diffusion model and ask whether this can fit the experimental data. We have then used the model to predict the spatial dynamics of calcium under conditions where imaging is not possible. In particular, we removed the indicator dye from the model to predict the calcium spatial dynamics in an unmanipulated cell. Our analysis shows that diffusion, without the operation of regenerative processes such as calcium induced calcium release, is likely to be responsible for transmission of calcium signals into the interior of these neurones. The nuclear envelope attenuates brief cytosolic calcium transients but does not prevent equilibration on a timescale of tens of milliseconds.

2. Methods

Twelve day old chick embryos were killed by cervical dislocation. Dorsal root ganglia were removed and incubated in 1.25 mg ml^{-1} collagenase (Sigma) for 3 h at 37 °C. Ganglia were then triturated and the cell suspension plated onto polyornithine coated (5 μg ml^{-1} for at least 1 h) plastic dishes in Dulbecco's modification of Eagles medium (DMEM) supplemented with 10% foetal bovine serum, 20 ng ml^{-1} 7S NGF (Gibco), 50 units ml^{-1} penicillin and 50 μg ml^{-1} streptomycin. The day before the experiment the cells were blown off the substrate by flow from a Pasteur pipette and replated in the same medium on polyornithine coated glass cover slips. This replating procedure generated a population enriched in neurones, most of which comprised only a simple shaped cell body with no neurites.

On the day of the experiment, Hoechst 33342 (Sigma) was added to the growth medium at 2 μM to label the nuclei and cells incubated for at least 30 min. Results from initial experiments without Hoechst 33342 did not yield detectably different results. The medium bathing the neurones was then exchanged for a simple salt solution comprising (mM) NaCl 120, $MgCl_2$ 1.2, KCl 5.5, $CaCl_2$ 1.8, CsCl 10, TEA-Cl 1.8, HEPES 10, glucose 25; pH 7.2. The extracellular medium also contained tetrodotoxin (TTX, Sigma) at 1 μM to allow the measurement of inward calcium currents uncontaminated by sodium currents, and BayK 8644 (racemic mixture, Calbiochem) at 5 μM to enhance calcium signals. Standard measurements of calcium spatial dynamics used cells patched at room temperature through pipettes filled with (mM) CsCl 125, HEPES 10, MgATP 4, pH 7.2; plus 100 μM each of the 10 kDa dextran conjugates of Oregon Green 488 BAPTA-1 (OGBD, calcium indicator) and tetramethylrhodamine (TRITCD, calcium insensitive marker) (Invitrogen). After 3 min in whole-cell configuration cells were depolarized from a holding voltage of −70 to +10 mV for 50 ms to evoke calcium influx through L and N type calcium channels [13]. Other experiments used different pipette solutions as described below. Neurones were simultaneously imaged on a Zeiss 510 confocal microscope using a 63× water immersion lens, numerical aperture 1.3, and pinhole apertures corresponding to 3 μm confocal slices. 12 bit acquisition was used throughout. Excitation and emission wavelengths were, respectively: Hoechst = 351 nm, 385–490 nm; OGBD, fluorescein and calcein = 488 nm, 505–570 nm; TRITCD and calcein red-orange = 543 nm, >570 nm. Imaging of cells labelled with multiple dyes was always sequential, that is, the preparation was only illuminated with light of one wavelength at any one time. Intensity values measured from areas within the cell were corrected for background signal by subtraction of values measured from outside the cell and were then typically divided by the initial signal to yield I/I_o traces. Imaging of cells without dye at the same microscope settings indicated that autofluorescence (that is, the additional signal from inside the cells over and above that from outside) was less than 0.1% of the resting OGBD signal.

The properties of OGBD as a calcium indicator are defined by its K_d and dynamic range, where the latter is the fluorescence of a solution of dye at saturating calcium divided by the fluorescence of the same concentration of dye at zero calcium. K_d and dynamic range values were measured *in vitro*, using confocal microscope settings identical to those used for cell measurements, in calcium buffers (Invitrogen) in the presence of an equal concentration of TRITCD. The two batches of OGBD used had K_d and dynamic range values of 1.833 μM, DR = 4.55 and 1.567 μM, DR = 4.65, respectively.